FOREWORD

1988—A Year of Discovery

Lunch on January 19, 1988 was an epiphany. Not because of the food, I hasten to add, which was indifferent deli fare. Rather because of the ideas that arose during the mealtime conversation with Vidal de la Cruz and Tom McCutchan of the Malaria Division at the National Institute of Allergy and Infectious Diseases.

I had just given a talk to the malaria group about what has now come to be called "phage display." Phage display had its genesis in May 1984, while I was working on filamentous bacteriophage during my sabbatical in Bob Webster's lab in the Biochemistry Department at Duke University. I'd been experimenting with pIII, one of the coat proteins, and it occurred to me that because of its domain structure, its surface location at one tip of the virion, and its apparent flexibility as evidenced in electron micrographs, the protein might tolerate being fused to foreign polypeptides without losing its function. It would be an unusual fusion protein, I realized, because it would be associated with the infectious particle itself. Conventional phage expression vectors like λgt11, in contrast, encode fusion proteins that aren't incorporated into the virion.

If the fused foreign polypeptides were indeed displayed on the surface of the filamentous phage, it probably would be accessible to antibodies. This raised the possibility that a large library of pIII fusions could be surveyed for polypeptides that bind a specific antibody using a powerful selection technique; affinity purification. First, the antibody would be tethered to a solid support; the phage library would then be passed over the support, allowing those few particles displaying an antibody-binding polypeptide to react with the antibody and thereby become attached to the support;

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after nonbinding particles had been washed away, the phage that remained—highly enriched for particles displaying antibody-binding polypeptides—would be eluted under conditions that didn't destroy the phage's infectivity. The eluted phage could then be cloned and propagated indefinitely by infecting fresh bacterial host cells. Affinity purification would make it easy to survey billions of clones *en masse*, whereas dozens of plaque-lifts had to be laboriously processed to survey just a million λgt11 clones. Paul Modrich, down the hall from Webster's lab, had just what I needed to test the idea: the gene for *EcoRI* restriction enzyme (a source of clonable gene fragments), antibody against the enzyme (to affinity-select clones displaying fragments of the enzyme), and vast quantities of biochemically pure enzyme (a competitive inhibitor to check the specificity of the antibody-phage reaction). It worked! I reported the first results at the Cold Spring Harbor phage meeting in August, 1984, and published a 1985 paper in *Science*. By the time I gave my talk to the NIAID malaria group three and a half years later, Steve Parmley, a grad student in my lab, had developed a practical display vector and a very effective affinity selection method.

I still thought of phage display mostly as an alternative to λgt11 and other conventional expression vectors, but a passing remark of McCutchan at that lunch utterly changed my outlook. He said (more or less): "You know, you could use phage to out-Geysen Geysen." I didn't know who he was talking about, but I soon found out. Mario Geysen had devised simple methods for synthesizing large numbers of peptides on pins in microtiter format. His "pepscan" technology is used widely for mapping the epitopes recognized by antiprotein antibodies. More importantly, he'd devised a clever way of searching through all possible octapeptide sequences to delineate an epitope for an antibody without any usable knowledge of its specificity. If synthetic oligonucleotides coding for all possible short peptides (hexamers, for instance) could be cloned into a display vector, you'd have a universal "epitope library" that might contain peptide ligands for almost any antibody. It would be easy to identify a ligand for any given antibody: simply use it to affinity-select the relevant clones by Parmley's technique. I saw that by making degenerate oligonucleotides, I could produce all the necessary coding sequences in a single run of the chemical synthesizer. It should be easy to make the universal library.

By the time I submitted my next NIH proposal five weeks later, the epitope library dominated my plans for the future. I imagined numerous immunological problems that might be approached with the new technology (if I succeeded in developing it). As it happened, Hannah Alexander, who had done much of the key work on protein epitopes in Richard Lerner's lab at Scripps, now worked in Columbia. She helped me through a crash course in the field, and forged an alliance with the Scripps group that was to prove invaluable when it came time to test the epitope library concept. The paper Parmley and I submitted to *Gene* in June 1988 outlined the concept, and in August, Shannon Flynn, an undergraduate, started synthesizing a miniature peptide library that contained (among many other sequences) the epitope for antibodies of known specificity from Lerner's group.

In October, 1988, while preparing to resubmit my NIH proposal (the previous one had failed), I happened to read the *Science* article by Bird *et al.* on single-chain

antibodies. I was inspired by a new vision. I imagined a universal library of single-chain antibodies displayed on phage. The antibodies would have one or a few framework sequences, but their CDRs (complementarity-determining regions) would be randomized to generate a vast diversity of binding specificities. The user could use any antigen of interest to affinity-purify clones whose displayed antibodies happened to bind that antigen. In this way, monoclonal antibodies might be obtained to almost any antigen by simple microbiological means, without the need for animals or animal cells in culture. I thought these artificial antibodies would eventually replace conventional and monoclonal antibodies for many purposes. The new idea, which I dubbed "infectious antibodies" (now called phage antibodies), became a key aspect of the new grant proposal I submitted to NIH on November 1, 1988.

The eventful year of 1988 closed appropriately with yet another broadening of my horizons. On December 15, I was sitting in Jim Larrick's office at Genelabs, Inc. "Drugs!" he said cryptically. He explained that if the epitope library were used to find new peptide ligands for receptors other than antibodies, these ligands would represent a new and broadly applicable route to drug candidates. It was so obvious! Yet, up to then my plans had been exclusively immunological—reflecting, I suppose, my doctoral and postdoctoral training in that field. From that day on, drug discovery was to become a new element in my concept of phage-display technology.

Work on the epitope library did not begin in earnest until the summer of 1989, both because I was occupied with other projects and because my lab was not funded between May 1988 and July 1989, when my new NIH grant—finally successful!—started. Two people came to the lab then; Jamie Scott, a postdoctorate student who constructed and tested our first epitope library, and has been my close collaborator ever since; and a new technician, Robert Davis, who carried out many of the key experiments and has managed my lab now for six years.

When I look back on my year of discovery, I'm struck—and a little embarrassed—at how parochial my vision of phage display was at the beginning, before my education at the hands of my fellow scientists. Phage-display technology, as we now understand it, and as is exemplified in this book, has been very much a communal invention. There's little sense in trying to apportion credit for such advances to individuals—for patent purposes or any other reason. But there is much sense, I think, in maintaining a vigorous, public community of scientists, talking freely with one another, whose ideas, excitement, and results are selected, amplified, recombined, tested, and reselected much as are the phage libraries.

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